

(19)日本国特許庁 (JP) (12) 公開特許公報 (A)

(11)特許出願公開番号  
特開2002-128690  
(P2002-128690A)  
(43)公開日 平成14年5月9日(2002.5.9)

(5)Int.Cl. <sup>7</sup>	識別記号	FI	チカラ <sup>7</sup> (参考)
A61K 38/00	101	A61P 29/00	101 4C084
A61P 29/00		35/00	
A61P 35/00		43/00	
43/00		A61K 37/02	
審査請求 未請求 請求項の数 2 OL (全 5 頁)			
(21)出願番号	特開2000-316464(P2000-316464)		
(22)公開日	平成12年10月17日(2000.10.17)		

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Fターム(参考)	4C084 A02 A03 B44 D416 DN25 M02 N405 ZB52 ZB262 ZC412 ZC752

(54)【発明の名称】 アポトーシス誘導剤

(57)【要約】  
【解決手段】 TNF- $\alpha$ 及びIL-4を有効成分とするアポトーシス誘導剤。  
【効果】 本発明アポトーシス誘導剤は、TNF- $\alpha$ 又はIL-4を単独で用いた場合に比べ、アポトーシス誘導効果が相乗的に増強され、副作用の少ない制癌剤、免疫調節剤、自己免疫疾患治療剤、肝臓、肝臓癌等の肝疾患治療剤等として使用できる。

【特許請求の範囲】

【請求項1】 TNF- $\alpha$ 及びIL-4を有効成分とするアポトーシス誘導剤。  
【請求項2】 悪性腫瘍又は慢性関節リウマチの予防・治療薬である請求項1記載のアポトーシス誘導剤。  
【発明の詳細な説明】

【0001】  
【発明の属する技術分野】 本発明はアポトーシス誘導剤に関するものである。  
【0002】

【従来の技術】 アポトーシスはプログラムされた細胞死の一形態であり、古典的細胞死(ネクローシス)と対比されるものである。アポトーシスは生化学上の種々の条件下に起こり、その形態学的特徴として、周囲の細胞との接触の欠乏、細胞膜の濃縮化、エンドスクレアーゼの活性化に関連したクロマチンの凝縮及び核膜崩壊、核の分断化、細胞表面の濃縮化の消失、細胞表面の平滑化(細胞表面の水形成: membrane blebbing)及びエンドヌクレアーゼによるDNAの断片化が観察され、アポトーシス細胞の最終断片が溶解する細胞により回収される機構として働いている (Bovall, E. and Wyllie, A. H., Immunology Today, 7 (4), 115-119 (1986); Science, 245, 301-305 (1989) )。

【0003】 アポトーシスは正常な発生・分化に不可欠な生理的細胞死であり、正常な生体組織の細胞回転などにおいて個々の細胞に起こっているが、癌性腫瘍、白血病、増殖性皮膚病、慢性関節リウマチ、自己免疫疾患等の疾患においては、アポトーシスが過剰に抑制され、その結果、細胞に損傷蓄積が生じるものと考えられている。例えば、フタナベ-アブナカからはMR L1 p r / l p rマウスにおいては、アポトーシスに阻害する Fas 分子に異常があり、胸腺における自己反応性T細胞のネガティブセレクション(アポトーシス)機構がうまく働かず、その結果自己免疫疾患が発症すると示唆されている (Watanabe-Fukunaga, K., et al., Nature, 356, 314-317 (1992) )。また、慢性肝炎が肝硬変、肝臓に移行していく過程では、アポトーシスは抑制状態にあり、これがサイトキニンT細胞による肝細胞の破壊に続く増殖化、肝硬変へと進展するものと考えられている。従って、斯かる疾患に罹患する細胞のアポトーシスを誘導する物質は、当該疾患の予防・治療薬として有用である。

【0004】 従来、蛋白質合成阻害剤であるシクロヘキシミドやRNA合成阻害剤であるアクチノマイシンD、腫瘍死因子(以下、「TNF- $\alpha$ 」)という)やリポホトキシン(LT)等のサイトカイン類にアポトーシスの誘導作用があることが報告され (Martin, S.J., et al., J. Immunol., 145, 1859-1867 (1990); Strelow, A., et al., J. Exp. Med., 192, 601-611 (2000))、また最近ではインターロイキン4 (以下、「IL-4」)という)

に、ヒト血球や好細胞に対してアポトーシス誘導作用があることが報告されている (J. Immunol., 148 (6), 1812-1816 (1992); J. Allergy Clin. Immunol., 102 (6 Pt. 1), 1013-1020 (1998))。

【0005】 しかし、これまでに知られている物質は、アポトーシス誘導活性や副作用の点から充分なものではなく、アポトーシス誘導効果が強く且つ安全性の高いアポトーシス誘導剤が求められていた。

【0006】  
【発明が解決しようとする課題】 本発明は、有効性が強く且つ安全性の高いアポトーシス誘導剤を提供することを目的とする。  
【0007】

【課題を解決するための手段】 本発明は、斯かる課題に鑑み、アポトーシス誘導活性を有する物質について鋭意研究した結果、TNF- $\alpha$ とIL-4を併用した場合に、未分化細胞や前駆体細胞に対してそれぞれが有するアポトーシス誘導効果が相乗的に増強され、アポトーシスの過剰抑制に伴う疾患の予防・治療薬として有用であることを見出し、本発明を完成した。

【0008】 すなわち、本発明は、TNF- $\alpha$ 及びIL-4を有効成分とするアポトーシス誘導剤を提供するものである。

【0009】  
【発明の発見の形態】 本発明のアポトーシス誘導剤は、TNF- $\alpha$ とIL-4を有効成分とするものであるが、ここで、TNF- $\alpha$ とは、炎症を過した生体防御機構を中心に、抗腫瘍作用、破骨作用、細胞への脂質の取り込み作用、インターロイキン-1やコロニー刺激因子の生産誘導作用等、多様な生物活性を示す分子重17 kDaのポリペプチドであり、IL-4とは、広い範囲の免疫細胞刺激作用(B細胞の形質細胞への分化、T細胞の分化増殖)を中心に、抗腫瘍作用、I型アレルギーの増進作用、抗炎症作用等を有するサイトカインの一種である。これらTNF- $\alpha$ 及びIL-4には前述したようにアポトーシス誘導作用があることが報告されているが、TNF- $\alpha$ とIL-4を併用した場合に、アポトーシス誘導効果が相乗的に増強されることが全く予測されることがなかったことである。

【0010】 本発明のアポトーシス誘導剤に用いられるTNF- $\alpha$ 及びIL-4としては、それぞれTNF- $\alpha$ 及びIL-4としての活性を有する、天然型或いは遺伝子組換えにより産生された組換え体の何れもが含まれる。

【0011】 天然型のTNF- $\alpha$ は、例えば、センダイウイルス (Sendai Virus) 株にヒトリンパ球株系A549細胞の基底の細胞株の培養上清より、アフリニティクロマトグラフィーやHPLCなどの既知方法に従って精製することにより得ることができ、遺伝子の組換えによって得られるTNF- $\alpha$ は、既知の遺伝子を用い

込んだプラスミド或いはベクターを導入した大腸菌や既存細胞株の生産装置を同時に精製することにより得ることが可能である。

【0012】また、天然のIL-4は、ヒトT細胞クロニンや未精化T細胞或いは任意の胚芽細胞株をマイトジエン等で非特異的に刺激した培養上清から同様に精製して取得でき、相対IL-4は胚芽細胞株の利用によって船配と同様にして得ることが可能である。

【0013】本発明のアポトーシス誘導剤は、TNF- $\alpha$ とIL-4とが同一の製剤中に含まれるように調製されてもよく、或いは、TNF- $\alpha$ とIL-4のそれぞれを別個の製剤として調製し、これら2つの製剤を使用するものでもよい。また、TNF- $\alpha$ とIL-4の配合比率は、アポトーシス誘導作用の相乗効果が発揮できれば特に限定されずそれぞれ1〜99%の範囲で混合されることができ、特にTNF- $\alpha$ を30〜70%、IL-4を70〜30%で配合することが好ましい。

【0014】このようにして調製された本発明のアポトーシス誘導剤は、後記実施例に示すようにTNF- $\alpha$ 又はIL-4を単独で用いた場合に比べ、アポトーシス誘導効果が著しく増進されるという相乗作用を發揮する。従って、TNF- $\alpha$ 又はIL-4を単独で投与する場合に比べて両者の投与量を大幅に減少させることができる。

【0015】本発明のアポトーシス誘導剤の成人に対する一日当たりの投与量は、広範囲に選択されるが、TNF- $\alpha$ については、通常一日当たり50 $\mu$ g/body〜500 $\mu$ g/body程度であり、IL-4については、一日当たり50 $\mu$ g/body〜500 $\mu$ g/body程度とするのが望ましい。

【0016】本発明のアポトーシス誘導剤は、その使用目的に応じ、医薬製剤としてこの分野で慣用されている各種の投与形態で使用される。所かる製剤は、通常使用される充填剤、増量剤、結合剤、付与剤、崩壊剤、緩衝剤、溶媒剤等の希釈剤或いは賦形剤を無毒性薬性担体として用いて調製される。剤形は、治療目的に応じて各種の剤形が選択でき、この代表的なものとして錠剤、丸剤、軟剤、液剤、懸濁剤、乳剤、顆粒剤、カプセル剤、坐剤、注射剤（液剤、製剤等）、点眼剤等が挙げられる。

【0017】錠剤の形態に成形するに際しては、相対としてこの分野で従来公知のものを広く使用でき、例えば乳糖、白糖、微細ナトリウム、ブドウ糖、尿素、デンプン、乳糖カルシウム、カオリン、結晶セルロース、ケイ酸等の賦形剤、水、エタノール、プロパノール、血シリップ、ブドウ糖液、デンプン液、ゼラチン液、カプセル、キシメチルセルロース、セラック、メチルセルロース、リン酸カルシウム、ポリビニルピロリドン等の結合剤、乾性デンプン、アルブミンナトリウム、カンテン素、ラミナラン素、乳糖水素ナトリウム、乳糖カルシウム、ポリ

病 (Idiopathic Thrombocytopenic Purpura: ITP)、自己免疫性血小板減少症、重症筋無力症、橋本病、インスリン依存型(1型)糖尿病等を例示できる。また、本発明のアポトーシス誘導剤は、骨髄異形性症候群、固形性血小核減少症、再生不良性貧血、突発性血小核減少症、乳がん、乳がん内臓型等の血小核減少を伴う各種の疾患、C型肝炎、B型肝炎、F型肝炎等の各種の肝炎、アルツハイマー病、アルツハイマー型老年痴呆症、心筋炎、ARDS (成人呼吸急迫症候群)、腎臓病、肝硬変、前立腺肥大症、子宮筋腫、気管支喘息、動脈硬化症、各種先天性奇形症、腎臓病、老人性白内障、慢性疲労症候群 (Chronic Fatigue Syndrome)、筋ジストロフィー (Myotonic dystrophy) 等の各種疾患にも適用可能である。

【0023】特に、本発明のアポトーシス誘導剤を賦形剤として用いる場合、その投与により癌細胞に対してアポトーシスを誘導でき、抗癌作用を發揮するが、これを癌の化学療法剤として知られている各種の抗癌剤や放射線療法と併用すれば、抗癌効果を一層増進させることができ、副作用の軽減を図ることができる。斯かる化学療法剤としては、例えば5-フルオロウラシル (5-FU)、協和製薬工業株式会社製)、マイトマイシン (Mitomycin、n.c. 同上社製)、フトラフル (FT-207、大塚製薬工業株式会社製)、エンドキサン (Endoxan、塩野義製薬株式会社製)、トヨマイシン (Toyomycin、武田薬品工業株式会社製) 等が挙げられる。

【0024】  
【実施例】以下に実施例を挙げて、本発明を更に詳細に説明する。

(1) 活細胞型毒性細胞リウマチ患者の骨髄細胞から、市販キット (Histopaque: Sigma社) を用いて、適度勾配法により単核球を分離した。分離した単核球から磁気ビーズ (Dynal CD34 progenitor cells selection system: Dynal社) を用いてCD34陽性細胞 (CD34+95%でCD19+8細胞は0.5%以下であった。【0025】(2) CD34+細胞を24ウェル平底マイクロプレート (No. 3596: Costar) に1.0 $\times$ 10<sup>5</sup>/ウェルになるようにSCF (10ng/ml) とGM-CSF (2ng/ml) を添加し、TNF- $\alpha$ 及びIL-4を併用した本発明のアポトーシス誘導剤は、TNF- $\alpha$ 又はIL-4を単独で用いた場合に比べ、死細胞率が相対的に増進することが示された。

試験材料	死細胞率 (P1陽性細胞) (%)			
	2日目	3日目	4日目	5日目
SCF/GM-CSF	11.3	11.3	6.3	6.3
SCF/GM-CSF	10.3	15.0	9.3	9.4
TNF- $\alpha$	10.1	10.3	8.0	8.3
TNF- $\alpha$ /IL-4	18.1	21.1	14.3	16.8

【0030】表2より、TNF- $\alpha$ 及びIL-4を併用した本発明のアポトーシス誘導剤は、TNF- $\alpha$ 又はIL-4を単独で用いた場合に比べ、死細胞率が相対的に増進することが示された。

(1ng/ml) を添加した培地で調製し、TNF- $\alpha$  (10ng/ml) 添加、IL-4 (10ng/ml) 添加、その両者 (10ng/ml+10ng/ml) を添加又は非添加 (対照) にして2週間培養した。培養後、PBSにて細胞を洗浄し、細胞を0.1% Triton X-100及び0.1% エンゲナトリウムを含むPBS200 $\mu$ lに浮遊し、P1染色 (10 $\mu$ g/ml Propidium Iodide) 10 $\mu$ lを添加し、4℃で10分後フローサイトメーター (EPICS XL: Coulter) にて免疫陽性細胞 (アポトーシス細胞) を検定した。結果を表1に示す。尚、培地は、RPMI-1640培地 (Life Technologies社) にペニシリン (100 unit/ml)、ストレプトマイシン (100 $\mu$ g/ml)、レグルタミン (0.3mg/ml) 及びFBS (10% Fetal bovine serum: Life Technologies社) を添加して用いた。また、SCF (stem cell factor)、GM-CSF (granulocyte-macrophage colony-stimulating factor)、TNF- $\alpha$ 及びIL-4は、いずれも市販品 (Peprotech社) を用いた。

【0026】  
【表1】

試験材料	死細胞率 (P1陽性細胞) (%)
対 照	2.23
TNF- $\alpha$	2.05
IL-4	4.03
TNF- $\alpha$ /IL-4	10.7

【0027】表1より、TNF- $\alpha$ 及びIL-4を併用した本発明のアポトーシス誘導剤は、TNF- $\alpha$ 又はIL-4を単独で用いた場合に比べ、死細胞率が相対的に増進することが示された。

【0028】実施例2  
HeLa細胞 (子宮頸癌扁平上皮癌由来ヒト培養細胞) を用い、実施例1 (2) と同様にして、TNF- $\alpha$ 及びIL-4の併用効果を試験した。尚、HeLa細胞は、2 $\times$ 10<sup>5</sup>/ウェルにて96ウェルプレートで培養した。結果を表2に示す。

【0029】  
【表2】

試験材料	死細胞率 (P1陽性細胞) (%)			
	2日目	3日目	4日目	5日目
SCF/GM-CSF	11.3	11.3	6.3	6.3
SCF/GM-CSF	10.3	15.0	9.3	9.4
TNF- $\alpha$	10.1	10.3	8.0	8.3
TNF- $\alpha$ /IL-4	18.1	21.1	14.3	16.8

【0031】  
【発明の効果】本発明のアポトーシス誘導剤は、TNF- $\alpha$ 又はIL-4を単独で用いた場合に比べ、アポトーシス誘導効果が相対的に増進され、副作用の少ない抗癌

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(5)

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8

和、慢性関節リウマチ治療剤、自己免疫疾患治療剤、肝炎、肝硬変等の肝疾患治療剤等として使用できる。

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## PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2002-128690  
(43)Date of publication of application : 09.05.2002

(51)Int.Cl.

A61K 38/00  
A61P 29/00  
A61P 35/00  
A61P 43/00

(21)Application number : 2000-318464 (71)Applicant : HIROHATA TOSHINARI  
(22)Date of filing : 17.10.2000 (72)Inventor : HIROHATA TOSHINARI

## (54) APOPTOSIS-INDUCING AGENT

## (57)Abstract:

PROBLEM TO BE SOLVED: To obtain an apoptosis-inducing agent consisting mainly of TNF- $\alpha$  and IL-4 as active ingredients.

SOLUTION: This apoptosis-inducing agent has a synergistically enhanced apoptosis-inducing effect as compared with the case of using the TNF- $\alpha$  or IL-4 singly and can be used as an anticancer agent, a chronic rheumatoid arthritis-treating agent, an autoimmune disease-treating agent and a treating agent for hepatic diseases such as hepatitis, hepatic cirrhosis, and exhibiting less adverse effects.

## LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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CLAIMS

[Claim(s)]

[Claim 1] The apoptosis inducer which makes TNF-alpha and IL-4 an active principle.

[Claim 2] The apoptosis inducer according to claim 1 which is a malignant tumor, or prevention and the remedy of rheumatoid arthritis.

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## DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to an apoptosis inducer.

[0002]

[Description of the Prior Art] Apoptosis is one gestalt of the programmed cell death, and is contrasted with classic cell death (necrosis). Apoptosis happens to the bottom of the condition of the versatility on physiology. As the morphological description Condensation and pyknosis of chromatin relevant to lack of contact into a surrounding cell, the inspissation of cytoplasm, and the activity of endonuclease. Nuclear segmentation, disappearance of the microvillus of cell surface, smoothening (blistering) of cell surface; membrane blebbing) of cell surface, and fragmentation of DNA by ENDONUCLEASE are observed. It is discussed as a device in which the englobement is carried out by the cell which the last fragment of an APOTIKU somatic cell adjoins (Duvall, E. and Wylie, A.H., Immunology Today, and 7 (4) --- 115-119(1986); Science, 245,301-305 (1989).

[0003] Although apoptosis is physiological cell death indispensable to normal generating and differentiation and it has happened to each cell in the cell kinetic of a normal body tissue etc., in diseases, such as a malignant tumor, leukemia, a growth sex skin disease, rheumatoid arthritis, and an autoimmune disease, apoptosis is controlled superfluously, consequently it is thought that a functional disorder arises into a cell. For example, WATANABE-FUKUNAGA and others is in the Fas molecule which participates in apoptosis about abnormalities in a MRL/lpr/lpr mouse, and the negative selection (apoptosis) device of the self-reactivity T cell in a thymus gland did not operate well, but it is suggested that the symptoms of an autoimmune disease develop as a result (Watanabe-Fukunaga, R., et al., Nature, 356,314-317 (1992)), moreover --- the process in which the chronic hepatitis shifts to liver cirrhosis and hepatic carcinoma --- apoptosis --- a control condition --- it is --- this --- a site --- an ibis --- it is thought that it progresses to the fibrosis and the liver cirrhosis following the inflammation of the hepatocyte by the chie T cell. Therefore, the matter which guides the apoptosis of a cell which participates in this disease is useful as prevention and a remedy of the disease concerned.

[0004] The actinomycin-D which is the cycloheximide and inhibitor of RNA synthesis which are the former and a protein synthesis inhibitor, it reports that cytokine, such as a tumor necrosis factor (henceforth "TNF-alpha") and lymphotoxin (LT), has an induction operation of apoptosis --- having (Martin, S.J., et al., J.Immunol., 145, and 1859-1867 (1990) ---) Strelow, A., et al., J.Exp.Med., 192, 601-611 (2000). Recently, moreover, to interleukin 4 (henceforth "IL-4") it is reported that there is an apoptosis induction operation to Homo sapiens monocyte or eosinophile leucocyte (J --- Immunol. and 148 (6) ---) 1812-1816 (1992), J.Allergy Clin.Immunol., 102 (6 Pt 1), 1013-1020 (1999).

[0005] However, the matter known until now was not enough from the point of apoptosis induction activity or a side effect, and the apoptosis inducer with high safety with high and apoptosis induction activity was called for.

[0006]

[Problem(s) to be Solved by the Invention] This invention aims to let effectiveness offer a high

apoptosis inducer with high safety.

[0007]

[Means for Solving the Problem] The apoptosis inductive effect which each has to an undifferentiated cell or a precursor cell was reinforced in multiplication, and this inventions completed a header and this invention for it being useful as prevention, and a remedy of the disease accompanying superfluous control of apoptosis, when IL-4 were used together with TNF-alpha, as a result of inquiring wholeheartedly in view of this actual condition about the matter which has apoptosis induction activity.

[0008] That is, this invention offers the apoptosis inducer which makes TNF-alpha and IL-4 an active principle.

[0009]

[Embodiment of the Invention] Although the apoptosis inducer of this invention makes TNF-alpha and IL-4 an active principle With TNF-alpha, focusing on the living body defense mechanism through inflammation here Antitumor action, An osteoclasia operation, the incorporation inhibitory action of the lipid to a cell, a production induction operation of interleukin 1 and a colony stimulating factor, etc., It is the polypeptide of molecular-weight 17kDa which shows various bioactive, and is a kind of the cytokine which has antitumor action, an l-beam allergy induction operation, anti-inflammatory activity, etc. focusing on the immunocyte stimulation (differentiation to the plasma cell of a B cell, differentiation growth of a T cell) of the large range in IL-4. As mentioned above, it is reported to these TNF-alpha and IL-4 that there is an apoptosis induction operation, but when IL-4 are used together with TNF-alpha, this apoptosis inductive effect's being reinforced in multiplication is that completely predicting became impossible.

[0010] any of the recombinant produced by the natural mold or gene recombination which has the activity as TNF-alpha and IL-4, respectively as TNF-alpha used for the apoptosis inducer of this invention, and IL-4 --- although --- it is included.

[0011] TNF-alpha of a natural mold can be obtained by refining according to the known approaches, such as affinity chromatography and HPLC, from the culture supernatant of the existing cell strains, such as Sendai Virus (Sendai Virus) stimulus Homo sapiens B lymphoblast stock BALL-1, and TNF-alpha obtained by recombination of a gene can be obtained by refining similarly the Escherichia coli and the production protein of the existing cell strain which introduced the plasmid or vector incorporating a known gene.

[0012] Moreover, by mitogen etc., a Homo sapiens T cell clone, a peripheral blood T cell, or the existing cell strain of arbitration is refined similarly, and can be acquired from un-stimulating or the stimulated culture supernatant, and natural IL-4 can obtain recombination IL-4 as well as the above by use of the existing gene.

[0013] The apoptosis inducer of this invention may be prepared so that TNF-alpha and IL-4 may be contained in single pharmaceutical preparation, or it may prepare TNF-alpha and each of IL-4 as separate pharmaceutical preparation, and may use these two pharmaceutical preparation together. Moreover, as for TNF-alpha and the rate of a compounding ratio of IL-4, it is desirable not to be limited especially if the synergistic effect of an apoptosis induction operation can be demonstrated, but to especially be mixed in 1 - 99% of range, respectively, to blend TNF-alpha and to blend IL-4 at 70 - 30% 30 to 70%.

[0014] Thus, the prepared apoptosis inducer of this invention demonstrates the synergism that apoptosis inductive effect is reinforced remarkably, compared with the case where TNF-alpha or IL-4 are independently used as shown in the after-mentioned example. Therefore, compared with the case where TNF-alpha or IL-4 are independently prescribed for the patient, both dose can be decreased sharply, and it becomes mitigable [ a side effect ].

[0015] Although the dose per [ to the adult of the apoptosis inducer of this invention ] day is chosen suitably broadly, it is usually 50microg/body per day - 50 mg/body extent about TNF-alpha, and it is desirable about IL-4 to consider as 50microg/body per day - 50 mg/body extent.

[0016] The apoptosis inducer of this invention is used according to that purpose of use with various kinds of administration gestalten commonly used in this field as physic pharmaceutical preparation. This pharmaceutical preparation is prepared using a diluent or excipients, such as

the bulking agent usually used, an extending agent, a binder, a \*\*\* agent, disintegrator, a surface active agent, and lubricant, as avirulent pharmacology support. Dosage forms can choose various kinds of gestalten according to the therapy purpose, and a tablet, a pill, powder, liquids and solutions, suspension, an emulsion, a granule, a capsule, suppositories, injections (liquids and solutions, suspension, etc.), ophthalmic solutions, etc. are mentioned as this typical thing. [0017] It faces fabricating in the gestalt of a tablet and a well-known thing can be conventionally used widely in this field as support. For example, a lactose, white soft sugar, a sodium chloride, grape sugar, a urea, starch, a calcium carbonate. Excipients, such as a kaolin, crystalline cellulose, and a silicic acid, water, ethanol, propanol, Simple syrup, grape-sugar liquid, starch liquid, a gelatin solution, a carboxymethyl cellulose, A shellac, methyl cellulose, potassium phosphate, the binder of polyvinyl-pyrrolidone sugar, Desiccation starch, sodium alginate, agar powder, the end of a laminaran. A sodium hydrogencarbonate, a calcium carbonate, and polyoxyethylene sorbitan fatty acid ester Sodium lauryl sulfate, a stearin acid monoglyceride, starch, Collapse inhibitors, such as disintegrator, such as a lactose, white soft sugar, stearin, cocoa butter, and hydrogenated oil, Absorption enhancers, such as a quarternary-ammonium-salt radical and sodium lauryl sulfate, Lubricant, such as a polyethylene glycol, etc. can be illustrated in adsorbents, such as moisturizers, such as a glycerol and starch, starch, a lactose, a kaolin, a bentonite, and a colloid silicic acid, purification talc, a stearate, and the end of a boric acid. Furthermore, a tablet can be used as the tablet which gave the usual coating if needed. for example, a sugar-coated tablet, a gelatin encapsulation lock, an enteric tablet, a film coated tablet or an auxiliary rim lock, and a multilayered tablet.

[0018] It can face fabricating in the gestalt of a pill, and a thing conventionally well-known in this field as support can be used widely, for example, disintegrator, such as binders, such as excipients, such as grape sugar, a lactose, starch, cacao butter, hardening vegetable oil, a kaolin, and talc, gumi arabicum pulveratum, powdered tragacanth, gelatin, and ethanol, and laminaran agar, etc. can be illustrated.

[0019] It can face fabricating in the gestalt of suppositories, and a conventionally well-known thing can be widely used as support, for example, the ester of a polyethylene glycol, cacao butter, higher alcohol, and higher alcohol, gelatin, semisynthetic glyceride, etc. can be mentioned.

[0020] When prepared as injections, liquids and solutions and suspension are sterilized, and it is desirable that they are blood and an isotonicity, and they can be faced fabricating in the gestalt of these liquids and solutions, an emulsion, and suspension, and can use all the things commonly used in this field as a diluent, for example, can mention water, ethyl alcohol, propylene glycol, ethoxylation isostearyl alcohol, polyoxy-ized isostearyl alcohol, and polyoxyethylene sorbitan fatty acid ester. In addition, the salt, the grape sugar, or the glycerol of sufficient amount to prepare an isosmotic solution in this case may be made to contain in physis pharmaceutical preparation, and the usual solubilizing agent, a buffer, an aponia-ized agent, etc. may be added. [0021] Furthermore, a coloring agent, a preservative, perfume, a flavor agent, a sweetening agent, etc. and other drugs may be made to contain if needed in this invention apoptosis inducer.

[0022] The apoptosis inducer of this invention obtained in this way can be applied to the various diseases resulting from superfluous control of apoptosis based on an apoptosis induction operation, and can expect the desired pharmacology effectiveness. As this application disease, for example Cancer, AIDS, ARC (AIDS associated diseases), ATL (adult T-cell leukemia: Adult T-cell leukemia), Hair Mr. cellularly leukemia (Hairy cell leukemia), the myelosis (HAM/TSP), HTLV-I associated diseases, such as respiratory disorder (HAB/HABA), arthrosis (HAAP), and uveitis (HAU), Collagen diseases, such as an autoimmune disease (systemic lupus erythematosus), for example, SLE, and rheumatoid arthritis (RA), Ulcerative colitis, Sjogren's syndrome, primary biliary liver cirrhosis, an outbreak thrombocytopenic purpura (idiopathic Thrombocytopenic Purapura:ITP), Autoimmune hemolytic anemia, myasthenia gravis, Hashimoto's disease, insulin-dependent (I-beam) diabetes mellitus, etc. can be illustrated. The apoptosis inducer of this invention Moreover, myelodysplastic syndromes, periodicity thrombocytopenia. Various kinds of diseases accompanied by thrombocytopenia, such as aplastic anemia, outbreak thrombocytopenia, and disseminated intravascular coagulation. Various kinds of hepatitis. such

as C mold, A mold, B mold, and a female mold, an Alzheimer disease, the Alzheimer mold senility Alzheimer's disease, Myocarditis, ARDS (adult respiratory urgency syndrome), an infectious disease, liver cirrhosis, prostatomegaly. It can be adapted also for various diseases, such as fibroid, bronchial asthma, arteriosclerosis, various congenital malformation, a nephritis, senile cataract, chronic fatigue syndrome (Chronic Fatigu Syndrome), and myotrophia dystonica (Myolonic dysrophy).

[0023] When using this invention apoptosis inducer as an anticancer agent especially, apoptosis can be guided to a cancer cell by the administration, a carcinostatic operation is demonstrated, but if this is used together with various kinds of anticancer agents and radiotherapy which are known as a chemotherapeutic drug of cancer, the carcinostatic effectiveness can be promoted further and mitigation of a side effect can also be aimed at. As this chemotherapeutic drug, 5-fluorouracil (5-FU, consonance fermentation industrial incorporated company make), a mitomycin (Mitomycin-C, shrine make same as the above), futrafur (FT-207, Taiho Pharmaceutical, Inc. make), endoxan (Endoxan, Shionogi& Co., Ltd. make), a toyomycin (Toyomicin, Takeda Chemical Industries, Ltd. make), etc. are mentioned, for example.

[0024]

[Example] An example is given to below and this invention is further explained to a detail. The monocyte was separated from an example 1(1) activity mold rheumatoid arthritis patient's bone marrow specimen by the concentration gradient method using the commercial kit (product made from Histopaque;Sigma). CD34 positivity cell (CD34+) was obtained from the separated monocyte using the magnetic bead (product made from Dynal CD34 progenitor cells selection system; Dynal). As for the cell which carried out separation recovery, the CD34+ cell of the CD19+ B cell was 0.5% or less at about 95%.

[0025] (2) Prepare by the culture medium which added SCF (10 ng/ml) and GM-CSF (1 ng/ml) so that it might become 1x10<sup>5</sup> / well to a flat bottom microplate (No.3596;Costar) 24 well about a CD34+ cell. TNF-alpha (10 ng/ml) addition, IL-4 (10 ng/ml) addition, and its both (10ng/ml+10ng/ml) were cultivated for two weeks by addition or un-adding (control group). The cell was washed in PBS after culture, the cell was floated to PBS200microl containing 0.1% TritonX-100 and 0.1% sodium citrate, and the dyeing positivity cell (apoptosis dead cell) was measured with PI dyeing (10microl of 10microl/ml Propidium iodide is added, and it is 10 minutes at 4 degrees C) back flow cytometer (EPICS XL-Coulter). A result is shown in Table 1. In addition, the culture medium added and used penicillin G (100 unit/ml), streptomycin (100mcg/ml), L-glutamine (0.3mg/ml), and FBS (10% product made from fetalbovine serum; Life Technologies) for RPMI-1640 culture medium (product made from Life Technologies). Moreover, the commercial item (product made from Pepro Tech EC) was used for each of SCF (stem cell factor), GM-CSF (granulocyte-macrophage colonystimulating factor), TNF-alpha, and IL-4.

[0026]

[Table 1]

培養材料	死細胞率 (T型性細胞%)
対 照	2.33
TNF-α	2.06
IL-4	4.03
TNF-α / IL-4	10.7

[0027] From Table 1, it was shown that the rate of a dead cell reinforces in multiplication compared with the case where TNF-alpha or IL-4 are independently used for the apoptosis inducer of this invention which used TNF-alpha and IL-4 together.

[0028] TNF-alpha and the combined effect of IL-4 as well as an example 1 (2) were examined using the example 2Hela cell (uterine cervix squamous-cell-carcinoma origin Homo sapiens cultured cell). In addition, the Hela cell was cultivated on the plate 96 well in 2x104/a well. A result is shown in Table 2.

[0029]

[Table 2]

試験材料	死細胞率 (P-I 陽性細胞) (%)			
	2 日目		3 日目	
	SGF/QM-CSF 無添加	SGF/QM-CSF 添加	SGF/QM-CSF 無添加	SGF/QM-CSF 添加
対照	7.3	11.5	6.3	6.9
TNF-α	10.2	15.0	9.3	9.4
IL-4	10.1	10.3	8.0	8.3
TNF-α/IL-4	18.1	23.1	14.3	18.8

[0030] From Table 2, it was shown that the rate of a dead cell reinforces in multiplication compared with the case where TNF-alpha or IL-4 are independently used for the apoptosis inducer of this invention which used TNF-alpha and IL-4 together.

[0031]

[Effect of the Invention] Compared with the case where TNF-alpha or IL-4 are used independently, apoptosis inductive effect is reinforced in multiplication and can use this invention apoptosis inducer as liver disease therapy agents, such as an anticancer agent with few side effects, a rheumatoid arthritis therapy agent, an autoimmune disease therapy agent, hepatitis, and liver cirrhosis, etc.

[Translation done.]